Clinical Investigation

Diurnal Rhythms of Cortisol, ACTH, and β-Endorphin Levels in Neonates and Adults

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To determine whether a diurnal rhythm exists in neonates admitted to neonatal intensive care units where there is continuous artificial lighting and periodic nursing and medical care, plasma cortisol, adrenocorticotropin (ACTH), and β-endorphin concentrations were measured in two groups of infants and in adult human volunteers. As expected, a diurnal rhythm was seen in adults. A diurnal rhythm was also found for cortisol and endorphin levels in neonates (3 to 4 days postnatally) with minimal stress and in infants who were clinically severely stressed. There was not a significant difference between the morning and afternoon concentrations of ACTH in these infants, but the afternoon concentrations were lower than the morning's, as would be expected. We found that a diurnal rhythm does exist in neonates within the first few days of postnatal life and that the continuous lighting and medical and nursing interventions do not interfere with this rhythm.

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The endogenous opiate, β-endorphin, is derived from proopiomelanocortin, the same precursor as for adrenocorticotropin (ACTH) and β-lipotropin.[1] Plasma concentrations of β-lipotropin and ACTH have been correlated with certain physiologic and clinical conditions and have shown a diurnal rhythm.[2,3] There is also evidence for a diurnal rhythmicity of β-endorphin and cortisol in human adults.[4,5]

In a number of clinical studies done in our laboratory, we have noticed an increase of plasma and cerebrospinal fluid β-endorphin concentrations during various pathophysiologic conditions.[6-9] During these studies the timing of blood collections was kept consistent because of the possibility of a diurnal rhythm. One report has suggested that a diurnal rhythm is a function of maturation and does not occur before 6 months of age.[10] Further, the infants in neonatal intensive care units are exposed to constant artificial light, and their sleep patterns are disrupted by periodic nursing and medical interventions.[11] Both of these factors may disrupt a biologic rhythm. Therefore, we measured the cortisol, ACTH, and β-endorphin concentrations from morning and afternoon blood specimens in three groups of human subjects to ascertain whether a diurnal rhythm existed in these infants.

Subjects and Methods

The study included 10 healthy adult volunteers and 20 neonates who were admitted to the neonatal intensive care unit of the University Hospital, Saskatoon, Saskatchewan, during a six-month period. The study was approved by the University of Saskatchewan Hospital Advisory Committee on Ethics in Human Experimentation, and informed parental consent was obtained before the study. The adult volunteers served as a control for the analytic techniques and previously proven diurnal rhythms.

The infants were divided into two groups according to the following criteria:

- Group 1 consisted of ten infants (5 male, 5 female) who were appropriate for their gestational ages. The gestational age ranged from 30 to 36 weeks (mean 33.4 ± 0.6 standard error of the mean) according to maternal dates and subsequently confirmed by gestational assessment. Postnatal ages were from 3 to 4 days (3.3 ± 0.2), and body weights were from 1,780 to 3,270 grams (2,359 ± 147.4). The infants were breathing spontaneously and had a diagnosis of mild hyaline membrane disease or wet lung disease (or both). All infants in this group showed minimal or no stress clinically as described previously.[12]
- Group 2 consisted of ten infants (5 male, 5 female) who were appropriate for gestational ages—28 to 37 weeks (31.7 ± 1.1)—at postnatal ages 3 to 4 days (3.3 ± 0.2), and who had body weights of 1,040 to 3,190 grams (1,846 ± 250). These infants suffered from severe hyaline membrane disease with or without apnea and were mechanically ventilated. They received intermittent chest physiotherapy and endotracheal suctioning and were considered clinically stressed.[11] Management of the infants was not altered for this study and was according to guidelines previously published.[13]

The adult volunteers (5 male and 5 female), making up group 3, ranged in age from 24 to 42 years (32.5 ± 1.7) and weighed between 52 and 78 kg (67.4 ± 2.6).

Blood specimens were collected by venipuncture or from

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ABBREVIATIONS USED IN TEXT
ACTH = adrenocorticotropin
CRF = corticotropin-releasing factor

an indwelling arterial line into tubes containing ethylenediaminetetraacetic acid as an anticoagulant at 9:30 AM and 3:30 PM. The specimens were immediately placed on ice and transferred to the laboratory where they were centrifuged (1,000*g* for 10 minutes). The plasma was carefully separated and frozen at −70°C until used for β-endorphin, ACTH, and cortisol assays.

Analysis

β-Endorphin was isolated from plasma in a manner similar to that previously described[6,14] using a Sephadex G-50 (superfine) column (Pharmacia Fine Chemicals). Plasma β-endorphin concentrations were measured by a radioimmunoassay purchased from New England Nuclear.[6] Lyophilized specimens were reconstituted in 1 ml of buffer (0.1 mol per liter phosphate buffer, pH 6.0).[6] Plasma ACTH and cortisol concentrations were also determined by radioimmunoassay using kits purchased from Radioassay Systems Laboratories Inc (Carson, California) and Diagnostic Products Corporations (Los Angeles), respectively. The antiserum has low cross-reactivities to other naturally occurring steroids. Adrenocorticotropin sensitivity, as defined by variations from the zero standard, was (±2 standard deviations) 0.4 pmol per liter. Cortisol sensitivity was 5.5 to 1,380 nmol per liter. Commercially available control specimens for cortisol were also assayed along with the specimens in the study. The calculated concentration for these control specimens was within the stipulated range. Duplicate measurements were obtained for each specimen. The intra-assay and interassay variations were 4.1% and 8.8%, respectively, for cortisol and 6.0% and 10.7% for ACTH.

Data Analysis

The data were analyzed by nonparametric paired comparison using the Wilcoxon's signed rank test in which morning readings were paired with the afternoon readings of the same persons. A *P* value of less than .05 was considered significant. All values are given as the mean ± 1 SEM.

Results

No significant differences existed in gestational ages, postnatal ages, or body weight in the infants assigned to groups 1 and 2.

The mean plasma morning (and afternoon) concentrations for cortisol, ACTH, and β-endorphin in group 1 were 124.0 ± 20.0 (89.4 ± 11.2) ng per ml, 10.6 ± 1.4 (8.8 ± 0.8) pg per ml, and 25.3 ± 3.5 (19.0 ± 2.9) pg per ml, respectively. The distribution for the concentrations of all three substances is shown in Figure 1. There was a statistically significant difference between morning and afternoon cortisol and β-endorphin concentrations. A significant difference was not seen for ACTH levels between the two collection periods ($P = .08$), although the PM concentrations were lower. Group 2 (Figure 2) morning (afternoon) concentrations of cortisol, ACTH, and β-endorphin were 179.3 ± 32.5 (146.0 ± 28.1) ng per ml, 9.1 ± 1.1 (8.5 ± 1.2) pg per ml, and 46.0 ± 8.2 (22.4 ± 3.6) pg per ml, respectively. A statistically significant difference was found between the morning and afternoon concentrations of the cortisol and β-endorphin concentrations in this group. As in group 1, the ACTH concentrations for group 2 were lower in the PM but not significantly ($P = .07$). There were no observable differences in values between male and female infants in either group nor was any relationship seen between responses and gestational age.

In the adult volunteers (group 3, Figure 3) all three substances revealed, as expected, a statistically significant difference between morning and afternoon concentrations. A diurnal rhythm was thus observed. The mean concentrations detected were as follows: cortisol, 190.7 ± 23.4 (127.0 ± 19.7) ng per ml; ACTH, 10.4 ± 1.2 (9.2 ± 1.1) pg per ml; and β-endorphin, 23.8 ± 3.0 (16.5 ± 2.1) pg per ml.

Discussion

Various biologic rhythms have been shown in humans and animals.[2,4] When the cycle of changes in concentrations is of 24 hours' duration, it is referred to as a circadian rhythm. Although diurnal and circadian rhythms are sometimes used interchangeably, the former is restricted to the variations of concentrations in the daylight hours.[15] This study has thus concerned itself with the diurnal rhythm because measurements were made during the daytime hours, although a circadian rhythm has been shown for these hormones and β-endorphin in adults. Ideally, more frequent blood specimen collections covering the 24-hour cycle would have been better, particularly because of the episodic nature of the secretion and release of these hormones. We measured the concentrations from two specimens mostly because of the convenience of collecting specimens and because we did not want to compromise the infants.

The concentrations recorded for cortisol, ACTH, and β-endorphin in the adult group showed that the 9:30 AM and 3:30 PM sampling times were suitable for showing a plasma variation related to a known biologic rhythm. These findings agree with those of Dent and co-workers, who showed that the highest cortisol and immunoreactive β-endorphin levels occur between 4 and 10 AM.[4] A similar pattern was seen for cortisol and β-endorphin concentrations in both groups of neonates participating in this study (Figures 1 and 2). Adrenocorticotropin concentrations did not differ significantly between the two collection periods in either group of neonates. This could partially be explained by the small sample size, the episodic nature of ACTH release, and by maturational differences. Food ingestion may also influence the secretory pattern of ACTH.[4,16(p131)] The half-lives of hormones vary in newborns, and this variation may indeed be more significant in sick premature neonates. There are also reports that ACTH concentrations do not always parallel plasma cortisol concentrations.[4,17,18] Adrenocorticotropin and β-endorphin are under control of the corticotropin-releasing factor (CRF). A similar pattern would therefore be expected for the two substances.

A linkage between ACTH and β-endorphin circadian secretion and CRF release has been confirmed in human and animal studies.[12] The CRF circadian rhythmicity is regulated by serotoninergic and cholinergic pathways that in turn are characterized by a circadian periodicity in their hypothalamic concentrations. Infants in group 1, although minimally stressed, were still sick. In other words, they cannot be classified as ideal control neonates. This fact may also account for the absence of a significant difference between the AM and

PM levels of ACTH. The fact that the concentrations of cortisol and β-endorphin showed a diurnal rhythm may indicate that these hormones have a stronger influence on self-preservation than does ACTH.

It is interesting that a diurnal rhythm for cortisol and β-endorphin was seen in these young neonates who are only 3 or 4 days of age because earlier reports, which dealt with children aged 2 months to 3 years of age, suggest that a typical circadian adrenocortical rhythm does not exist at these ages.[19-21] The heterogeneity of the populations in the studies by Onishi and associates, Franks, and Beitins and colleagues could account for their results.[19-21]

A diurnal rhythm was evident in the infants of group 2 despite the fact they were ill and stressed. Cortisol and β-endorphin levels were higher than those seen in the minimally stressed neonates (group 1). An elevated release of β-endorphin in stressed neonates has been reported previously.[6-8]

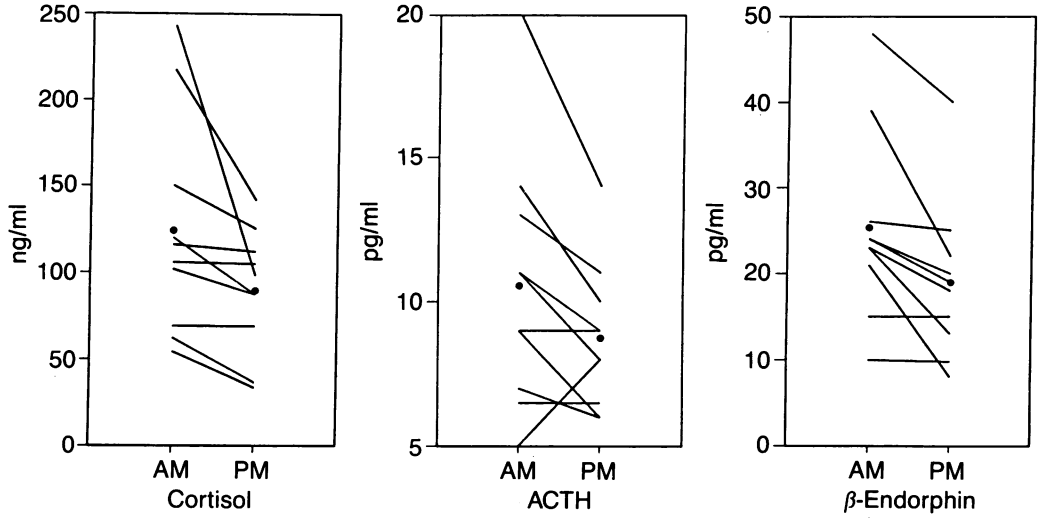

Figure 1.—Morning and afternoon concentrations of cortisol, adrenocorticotropin (ACTH), and β-endorphin are shown for 10 minimally stressed neonates. • = mean

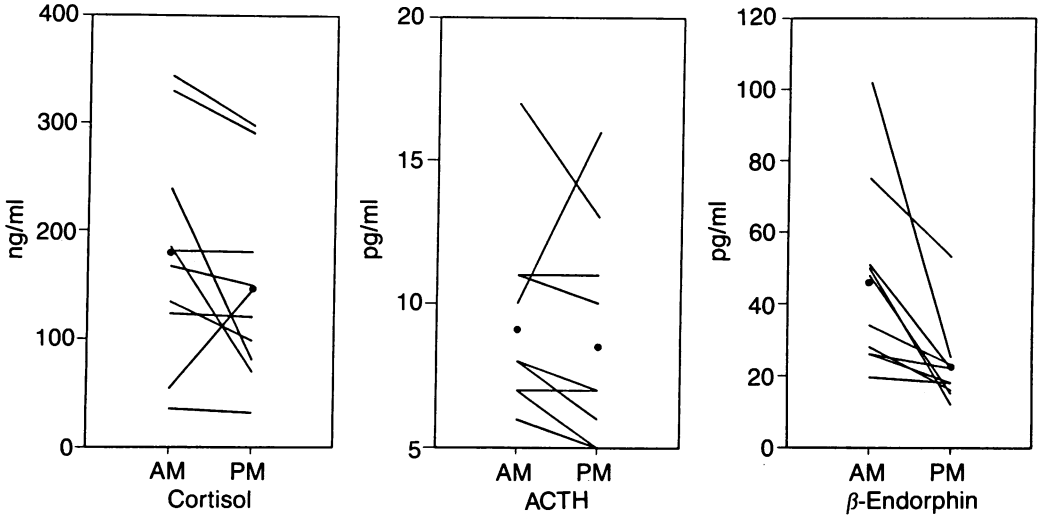

Figure 2.—Morning and afternoon concentrations of cortisol, adrenocorticotropin (ACTH), and β-endorphin are shown for 10 clinically stressed neonates. • = mean

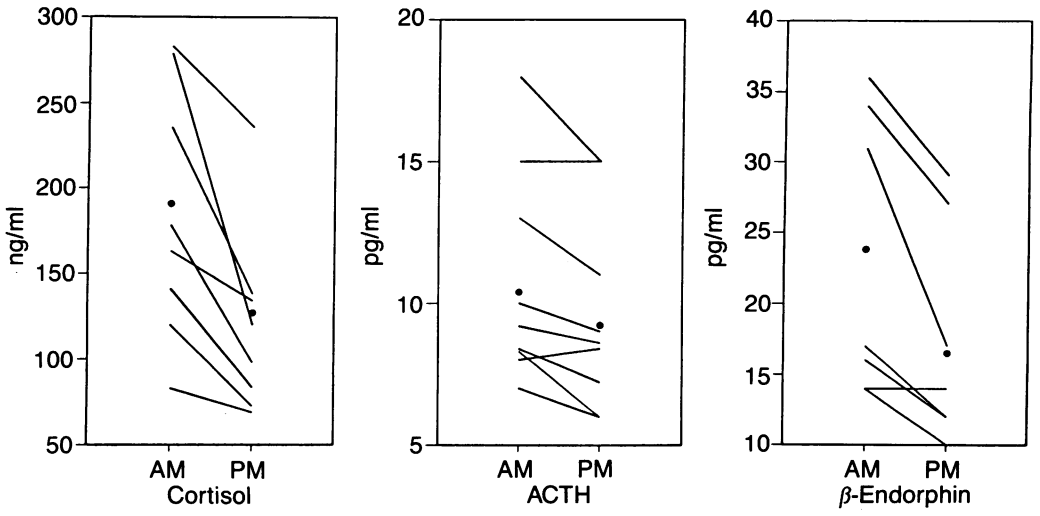

Figure 3.—Morning and afternoon concentrations of cortisol, adrenocorticotropin (ACTH), and β-endorphin are shown for 10 healthy adult human volunteers. • = mean

It would appear from this study that a diurnal rhythm exists in neonates in their first few days of life who are subjected to continuous artificial lighting and the care of a neonatal intensive care unit. This supports a study in adults by Dent and co-workers that indicated that the diurnal rhythm of immunoreactive β-endorphin does not appear to be closely associated with a specific sleep stage or to be related to the occurrence of sleep.[4] Our findings, however, do not agree with those of previous studies done of infants.[10,19] We fully recognize that specimens collected at only two specific time intervals may not be enough to confirm the existence or absence of a biologic rhythm, as the release of these hormones is episodic and depends on various other factors within a host. Further studies are needed to confirm these observations.

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